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A revision of recent taxonomic changes to the eyelash palm pitviper, *Bothriechis schlegelii* (Serpentes, Viperidae)

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Abstract

The taxonomy of the *Bothriechis schlegelii* species group has recently been expanded, nearly doubling the number of recognized species within this genus. However, the validity of these new species, defined primarily by mitochondrial DNA and without a robust evaluation of nuclear DNA and morphological data, warrants critical reassessment. This study re-examines the integrative data used in the initial analysis and employs additional visualizations of the genetic and morphological datasets. The results suggest that the genetic differences previously interpreted as species boundaries may instead reflect clinal variation, not independent lineages. Furthermore, DELINEATE species delimitation analysis and morphological assessments challenge the distinction of the proposed taxa. The findings indicate that only three species in the *B. schlegelii* group should be maintained as valid (*B. nigroadspersus*, *B. schlegelii*, and *B. supraciliaris*), while most taxa proposed by Arteaga et al. (2024) should be considered junior synonyms, or at most, as subspecies. This has significant implications for conservation priorities and resource allocation. In highlighting the need for a cautious and thorough approach to species delimitation, this study contributes to the broader discussion of taxonomic practices, the issue of taxonomic inflation, and their impact on biodiversity conservation.

Resumen

La taxonomía del grupo de especies *Bothriechis schlegelii* se ha expandido recientemente, casi duplicando el número de especies reconocidas dentro de este género. Sin embargo, la validez de estas nuevas especies, definidas principalmente por ADN mitocondrial y sin una evaluación robusta de datos nucleares, requiere una reconsideración crítica. Este estudio reexamina los datos integrativos utilizados en el análisis inicial y empleando visualizaciones adicionales de los conjuntos de datos genéticos y morfológicos. Los resultados sugieren que las diferencias genéticas previamente interpretadas como límites de especies podrían en cambio reflejar variación clinal, no linajes independientes. Además, el análisis de delimitación de especies DELINEATE y las evaluaciones morfológicas desafían la distinción de los taxones propuestos. Los hallazgos indican que solo tres especies del grupo *B. schlegelii* deben mantenerse como válidas (*B. nigroadspersus*, *B. schlegelii* y *B. supraciliaris*), mientras que la mayoría de los taxones propuestos por Arteaga et al. (2024) se consideran como sinónimos, o máximo, como subespecies. Esto tiene implicaciones significativas para las prioridades de conservación y la asignación de recursos. Al resaltar la necesidad de un enfoque cauteloso y exhaustivo para la delimitación de especies, este estudio contribuye a la discusión más amplia sobre las prácticas taxonómicas, el problema de la inflación taxonómica y su impacto en la conservación de la biodiversidad.

Kurzfassung

Die Taxonomie der *Bothriechis schlegelii*-Artengruppe wurde kürzlich erweitert, wodurch sich die Anzahl der anerkannten Arten innerhalb dieser Gattung nahezu verdoppelt hat. Die Gültigkeit dieser neuen Arten, die hauptsächlich durch mitochondriale DNA definiert wurden, und aber ohne eine robuste Bewertung nuklearer Daten auskommen, bedarf jedoch einer kritischen Neubewertung. Diese Studie untersucht die integrativen Daten, die in der ursprünglichen Analyse verwendet wurden, und nutzt zusätzliche Visualisierungen der genetischen und morphologischen Datensätze. Die Ergebnisse deuten darauf hin, dass die genetischen Unterschiede, die zuvor als Artgrenzen interpretiert wurden, stattdessen klinale Variationen und keine unabhängigen Linien darstellen könnten. Darüber hinaus stellen DELINEATE-Artabgrenzungsanalysen und morphologische Bewertungen die Unterscheidung der vorgeschlagenen Taxa in Frage. Die Ergebnisse zeigen, dass nur drei Arten in der *B. schlegelii*-Gruppe als gültig anerkannt werden sollten (B. nigroadspersus, *B. schlegelii* und *B. supraciliaris*),

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während die meisten von Arteaga et al. (2024) vorgeschlagenen Taxa als jüngere Synonyme oder höchstens als Unterarten betrachtet werden sollten. Dies hat erhebliche Auswirkungen auf die Prioritäten im Naturschutz und die Ressourcenzuweisung. Indem die Notwendigkeit eines vorsichtigen und gründlichen Ansatzes zur Artabgrenzung hervorgehoben wird, trägt diese Studie zur breiteren Diskussion über taxonomische Praktiken, das Problem der taxonomischen Inflation und deren Auswirkungen auf den Biodiversitätsschutz bei.

Key Words

conservation priorities, taxonomic inflation, species delimitation

Palabras clave

Inflación taxonómica, delimitación de especies, prioridades de conservación

Schlüsselwörter

Artenabgrenzung, Naturschutzprioritäten, taxonomische Inflation

Introduction

The delimitation of species has evolved significantly over time, transitioning from morphology-based approaches to molecular-based methods. With the advent of DNA sequencing, particularly the rapid amplification of mitochondrial DNA (mtDNA), there has been a surge in species delimitation based on genetic data (Hebert et al. 2003; DeSalle et al. 2005; Ahrens 2024). However, this shift has also brought challenges and potential pitfalls (Hillis 2019; Ahrens 2024).

Species delimitation is a complex process that should not rely solely on a single type of data, such as mtDNA. While mtDNA can provide valuable insights, it often leads to the over-splitting of species due to deep intraspecific genetic divergences, reduced gene flow, lack of recombination, incomplete lineage sorting, sex-biased dispersal, and asymmetrical introgression (Funk et al. 2003; Hebert et al. 2003; DeSalle et al. 2005). These issues can obscure the true nature of species boundaries. Therefore, an integrative approach that includes nuclear DNA, morphological data, and ecological information is necessary for accurate species delimitation (Padial et al. 2010). Integrating these methods can lead to a more accurate reflection of evolutionary history and avoid the pitfalls of premature taxonomic changes based solely on mitochondrial genetic data.

Recent studies on North American snakes that claim to use an integrative taxonomic approach have, in fact, made taxonomic changes based on mtDNA clades alone, with nuclear or morphological data playing a minimal role in diagnosing supposedly new species (e.g., Krysko et al. 2016; Carbajal-Márquez et al. 2020; Jadin et al. 2020; Tepos-Ramírez et al. 2021—but see Folt et al. 2019 for an example of articles fixing some of the issues).

To illustrate these challenges, the case of the palm pit vipers (genus *Bothriechis*) is particularly instructive. *Bothriechis* species have long captivated the fascination of both professional herpetologists and enthusiasts, taking on an almost mythic status due to their enigmatic behaviors, remarkable chromatic diversity, and potent venom. *Bothriechis* was historically composed of 11 species that mostly occur at relatively high elevations in mesic environments from Oaxaca to Ecuador and Venezuela (Campbell and Lamar 2004; Wylie and Grünwald 2016). The eyelash pit viper, *Bothriechis schlegelii* (Berthold, 1846) sensu lato, celebrated for its kaleidoscopic color variations, is particularly renowned. This species is distributed across a vast expanse of over 3,000 km, encompassing a myriad of eco-geographic zones, leading to the hypothesis that it might actually comprise multiple distinct species (Hurtado-Gómez 2009).

In a recent, exquisitely illustrated study, Arteaga et al. (2024) undertook a comprehensive taxonomic review of *Bothriechis schlegelii*. Employing an integrative approach, they analyzed morphological characteristics and molecular markers, including both nuclear and mitochondrial DNA sequences. Their work defined five novel species and reinstated three others previously subsumed under *B. schlegelii*. These new species came as a surprise, as these taxonomic findings nearly doubled the number of species in the genus *Bothriechis*. According to their findings, the nominal species is now geographically confined to Colombia's Cordillera Central (Fig. 1). Of the newly described and resurrected species, four are endemic to Colombia (*B. khwargi*, *B. klebbai*, *B. rasikusumorum*, and *B. schlegelii*), two occupy exclusive ranges within Ecuador (and possibly Peru, *B. hussaini and B. nitidus*), one straddles Ecuador and Colombia (*B. rahimi*), another between Colombia and Panama (*B. torvus*), and one spans from the Panama-Colombia border to southern Mexico (*B. nigroadspersus*). From now on, I refer to the species described or resurrected by Arteaga et al. (2024) as the *B. schlegelii* species group.

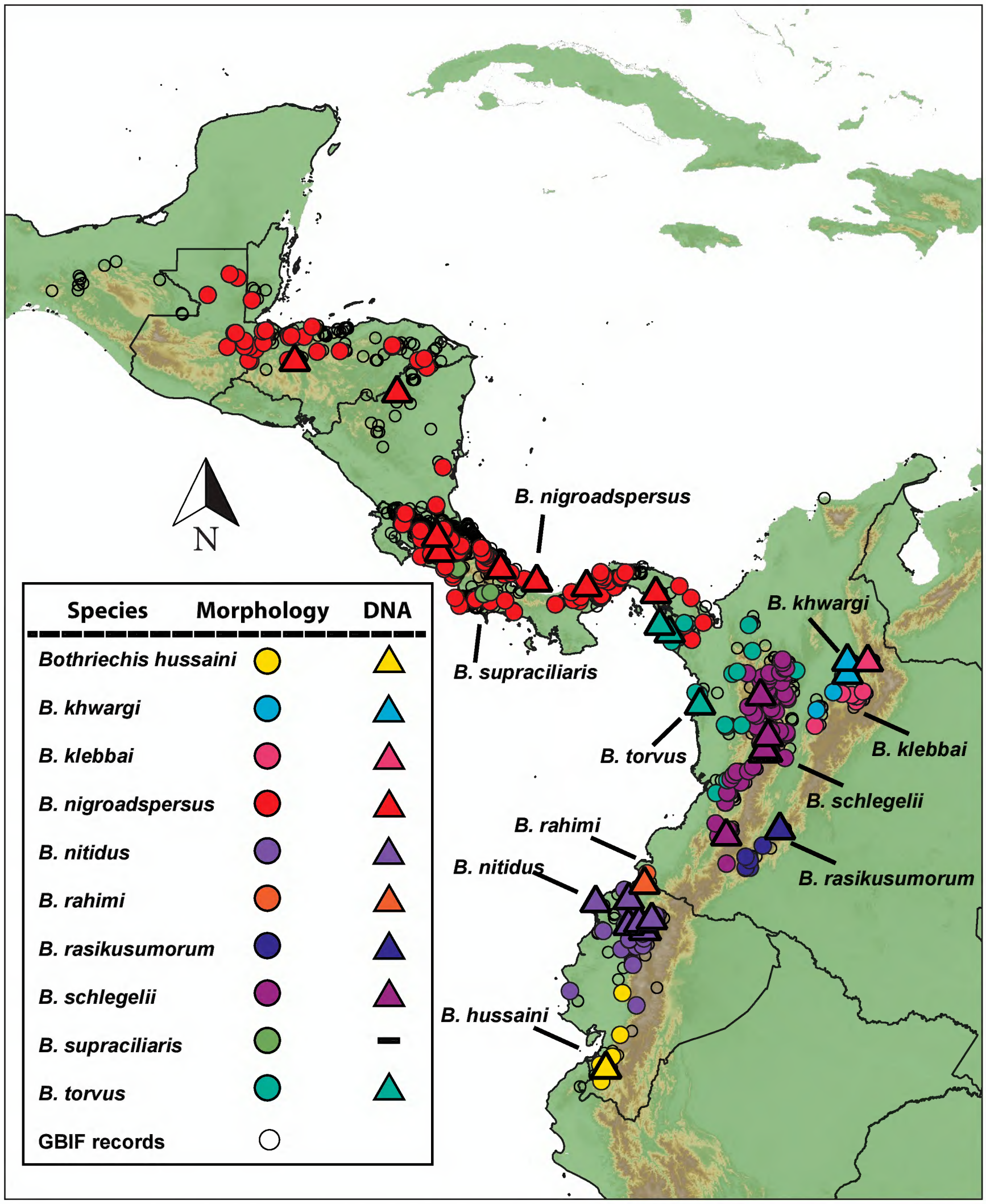


Figure 1. Geographic distribution map of the *Bothriechis schlegelii* species group. Colored circles indicate individuals assessed in Arteaga et al.'s morphological analysis. Colored triangles mark individuals for which molecular sequence data and locality information are available. Open circles correspond to occurrences from the Global Biodiversity Information Facility (GBIF) database.

Considering their molecular findings and methodology, questions arise regarding whether the newly described or resurrected taxa in fact represent separate species or if they only represent mitochondrial intraspecific diversity and not necessarily separate species within this complex. Herein, I reassess their datasets, employ other visualization methods on their genetic and morphological datasets, and re-evaluate the species designations based on their own rationale for the definition of species-level candidate taxa to rigorously test the species boundaries within the *Bothriechis schlegelii* complex.

Materials and methods

Molecular analyses

To re-evaluate the taxonomic conclusions of Arteaga et al. (2024), I first retrieved the same sequences that they analyzed in their study (Arteaga et al. 2024—supplementary table S1). They used seven loci: two nuclear (RAG1 and NT3), as well as five mitochondrial, including two non-coding (12S and 16S) and three coding (COI, Cyb7, and ND4) loci. Despite their extensive sampling of loci,

only three mitochondrial loci had less than 50% of missing data (16S, Cytb, and ND4), while their two nuclear loci had ~90% missing data. Each locus was aligned separately in MAFFT version 7 (Katoh et al. 2019) using the Q-INS-I option. The alignments were then concatenated using FASconCAT v.1.04 (Kück and Longo 2014). The final alignment comprised 4,959 base pairs, which included sequences from 88 representatives of the genus *Bothriechis*, as well as *Tropidolaemus wagleri* used as an outgroup.

For Bayesian phylogenetic analysis (BI), I used the same model for nucleotide substitution for each gene segment and codon position as presented by Arteaga et al. (2024, table 3). Subsequently, BI was executed through Mr. Bayes v3.2.2 (Ronquist et al. 2012) using the CIPRES science gateway (Miller et al. 2011). This involved four separate runs with a total of 10 million generations each, sampled every 1,000 generations. This included three hot chains and a single cold chain. I assessed the convergence of the runs by examining the overlap in likelihoods and parameter estimates, as well as the effective sample sizes and the potential scale reduction factor (PSRF), using Tracer v1.7.1 (Rambaut et al. 2022). The runs converged after 200,000 generations, which allowed for the initial 25% of the data to be excluded as burn-in. The remaining trees were combined using TreeAnnotator (Drummond and Rambaut 2007) and viewed using Figtree v1.4.2 (Rambaut 2014). Additional analyses, which included only the nuclear loci and only mtDNA data, were carried out the same way as previously described.

Maximum likelihood analyses (ML) were carried out on the combined data sequence in IQ-TREE (Nguyen et al. 2015) through its online platform (Trifinopoulos et al. 2016). An automatic model selection was used, and the analysis included 1,000 bootstrap iterations to determine support levels.

Due to the resemblance of the trees derived from ML and BI methods, I present only the phylogenetic tree resulting from BI of concatenated data, while the ML trees and mtDNA analyses are included as Suppl. material 3: figs S1–S3.

SplitsTree

I employed SplitsTree (Huson and Bryant 2006) to generate phylogenetic networks for the combined Cytb and ND4 mitochondrial DNA sequences of Arteaga et al. (2024). SplitsTree is a tool that generates phylogenetic networks from molecular data, which can reveal complex evolutionary relationships or homoplasy in the data not captured by traditional trees, thereby offering deeper insights into species divergence.

Genetic distances

Arteaga et al. (2024) highlighted sequence divergence as a critical factor for delineating new species, specifically using a 4% divergence threshold in the mitochondrial Cytb gene. Reexamining this threshold, I calculated pairwise genetic distances in the mitochondrial Cytb and ND4 genes using MEGA X software (Kumar et al. 2018). These analyses considered all codon positions and incorporated both nucleotide substitutions and variations in substitution rates, the latter modeled with a Gamma distribution.

Species delimitation analysis

Recent studies have shown that species delimitation analyses delimit populations and not necessarily species (Sukumaran and Knowles 2017; Chambers and Hillis 2020; Sukumaran et al. 2021), therefore, they can be misleading when defining species and tend to over-split species in the case of geographically widespread taxa (Chambers and Hillis 2020). In order to test for "species" (or populations) limits in *Bothriechis*, I employed the program DELINEATE (Sukumaran et al. 2021), which uses a protracted speciation model (PSM). DELINEATE allows the user to specify what lineages are considered as species validated with other types of data (morphology, ecology, etc.) and to test if certain molecular lineages could be considered species based on previous taxonomic cutoffs.

I set up a StarBeast3 configuration file using the program BEAUTI, part of the BEAST software package (Drummond and Rambaut 2007). Due to computational constraints, I used one individual per species, with the exception of *Bothriechis nigroadspersus*, in which I used one individual of each of the three major mitochondrial clades of Arteaga et al. (2024). I included the mitochondrial loci 12S, 16S, Cytb, and ND4, with a single strict clock model of substitution across genes and an HKY+G substitution model. The BEAST analysis ran for 200 million generations, sampling every 10,000 generations, for a total of 20,000 trees.

In order to check if the BEAST2 analysis had run for long enough, I examined the overlap in likelihoods and parameter estimates, as well as the effective sample sizes and the potential scale reduction factor (PSRF), using Tracer v1.7.1 (Rambaut et al. 2022). I then used Tracer v. 1.7.1 (Rambaut et al. 2022) to discard the first 10% of trees as burn-in. The remaining trees were combined using the Maximum Clade Credibility tree option in TreeAnnotator (Drummond and Rambaut 2007). I then prepared the population assignment input file for DELINEATE and constrained all species of *Bothriechis*, with the exception of the eight taxa described or resurrected by Arteaga et al. (2024).

Revision of morphological characters

Re-valuation of morphological characters and principal component analyses

In order to better visualize variation in the morphological characters of the new species of *Bothriechis* presented by Arteaga et al. (2024), I performed a principal component analysis (PCA) on the data they provided in their Suppl.

material 2: table S1. First, I excluded individuals that had more than 20% missing morphological character data. Separate analyses were conducted for scale count data and measurement data for both males and females.

For scale count data, I used 78 males and 95 females. For measurement data, I used two different standardization methods: standardization by snout-vent length (SVL) and regression residuals (Thorpe 1975, 1983). The number of individuals used in these analyses varied due to the specific requirements of each method. For males, I used 65 individuals for the SVL-standardized data and 67 for the regression residuals, for females, I used 84 individuals for the SVL-standardized data and 85 for the regression residuals.

I performed PCA in Python using multiple computing libraries: Pandas for data manipulation, NumPy for numerical computations, Scikit-learn for applying PCA, and Matplotlib for data visualization. The analysis began with preprocessing the dataset to exclude individuals with more than 20% missing data and standardizing the features according to the three methods mentioned above. PCA was then conducted to reduce the dimensionality of the data, extracting principal components that capture the most significant variance and patterns within the morphological traits.

Results

Molecular phylogenetic analysis

Concatenated analysis

The resulting phylogenetic relationships within *Bothriechis* inferred using BI were almost identical to those of Arteaga et al. (2024), which is not surprising, as the same samples and loci were used (Fig. 2). However, a number of differences should be pointed out. In Fig. 2 of Arteaga et al. (2024), it was claimed that the support values on intra-specific branches were not shown for clarity, however, they did show the support for some of the branches but not others. In the present study, the sister relationship between *B. rasikusumorum* and all other members of *B. schlegelii* was recovered with low posterior support (pp = 0.58), as well as *B. rahimi*, sister to *B. hussaini* + *B. nitidus*. All other species and relationships, despite being recovered as monophyletic (with one exception), were recovered with low support, which coincides with the branches not reported by Arteaga et al. (2024). The relationships between *B. klebbai*, *B. khwargi*, *B. torvus*, and *B. schlegelii* formed a polytomy. An individual of *B. khwargi* (UIS-R2529) was recovered as nested within *B. klebbai*. This was not an error when making the alignment, as when the sequence was blasted in GenBank, the closest matches were individuals of *B. klebbai*, however, it could be an issue that occurred when the authors submitted their sequences to GenBank.

In the ML analysis, the monophyly of South American *Bothriechis* was recovered with high support (bootstrap support (bs) = 100%), while *B. khwargi* was recovered as sister to all other species described or resurrected by Arteaga et al. (2024), but with low support (bs = 60%). *Bothriechis torvus* was recovered as sister to all remaining species, however, most nodes had very low bootstrap values, with the exception of *B. rahimi*, sister to *B. hussaini* + *B. nitidus*, which had relatively high support (bs = 98%). Once again, an individual of *B. khwargi* (UIS-R2529) was recovered as nested within *B. klebbai* (Suppl. material 3: fig. S1).

mtDNA analysis

Both ML and BI analysis of the concatenated mtDNA data resulted in a very similar topology to the concatenated phylogenies, which included nuclear loci (Suppl. material 3: figs S2, S3). This is no surprise, as the vast majority of the data in the mtDNA-nuclear concatenated analysis corresponds to mtDNA sequences.

Nuclear analysis

Of the individuals of *Bothriechis* sequenced by Arteaga et al. (2024), only 12 individuals from 5 species had nuclear DNA data, which limits its utility for estimating relationships within the group. The Bayesian inference analysis of the concatenated nuclear loci produced a poorly supported phylogeny in which none of the species formed a monophyletic group (Fig. 3). Most nodes in the phylogeny were poorly supported (posterior probability (pp) < 0.5), with only a handful of nodes with values higher than 0.5 pp (Fig. 3).

SplitsTree phylogenetic networks

The resulting phylogenetic networks from SplitsTree showed that many of the members of the *B. schlegelii* group had very low levels of genetic differentiation, with the genetic differentiation between species in that group being even lower than between populations of *B. nigroadspersus* (Fig. 4).

Genetic distances

Upon reevaluating the Cytb mitochondrial data presented by Arteaga et al. (2024), it was observed that within the *B. schlegelii* group, only four species exhibited an average genetic distance exceeding 4% in relation to all other members (as shown in Table 1). Arteaga et al. (2024) classified individual clusters as distinct species when their average pairwise (p) genetic distance was greater than 4%. They justified this cutoff by noting its applicability to species delineation in other *Bothriechis* taxa. Yet, such a level of interspecific divergence seems exclusive to *B. lateralis* vs. *B. guifarroi*, where other pairwise comparisons within the genus show divergences ranging from 6.3% to 20.3% in the Cytb gene, and only four comparisons yielded a divergence of less than 7% (Table 1).

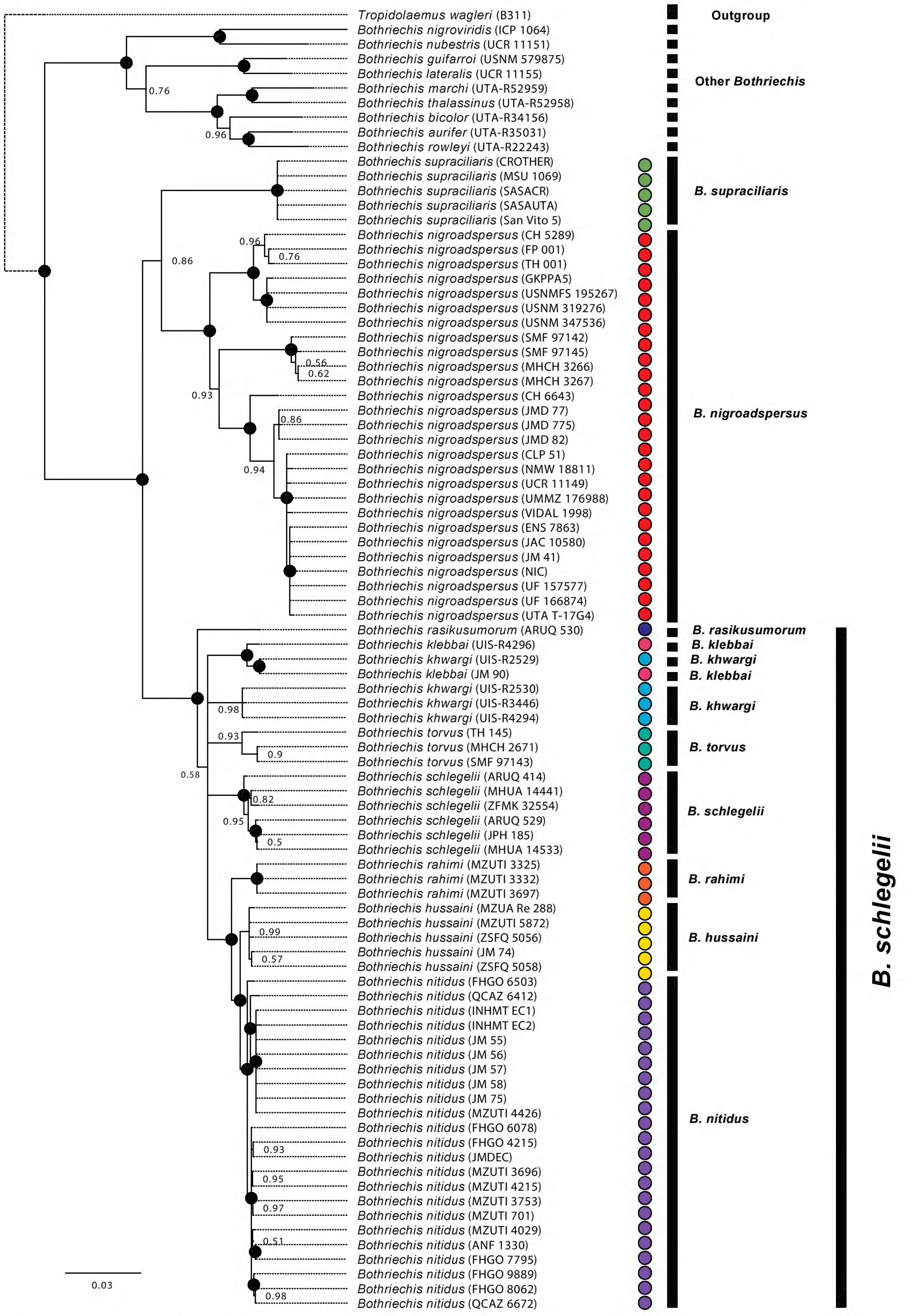


Figure 2. Phylogenetic relationships of *Bothriechis* species based on Bayesian inference from concatenated nuclear and mitochondrial loci. Node numbers indicate posterior support values, with black circles denoting posterior support of 1.

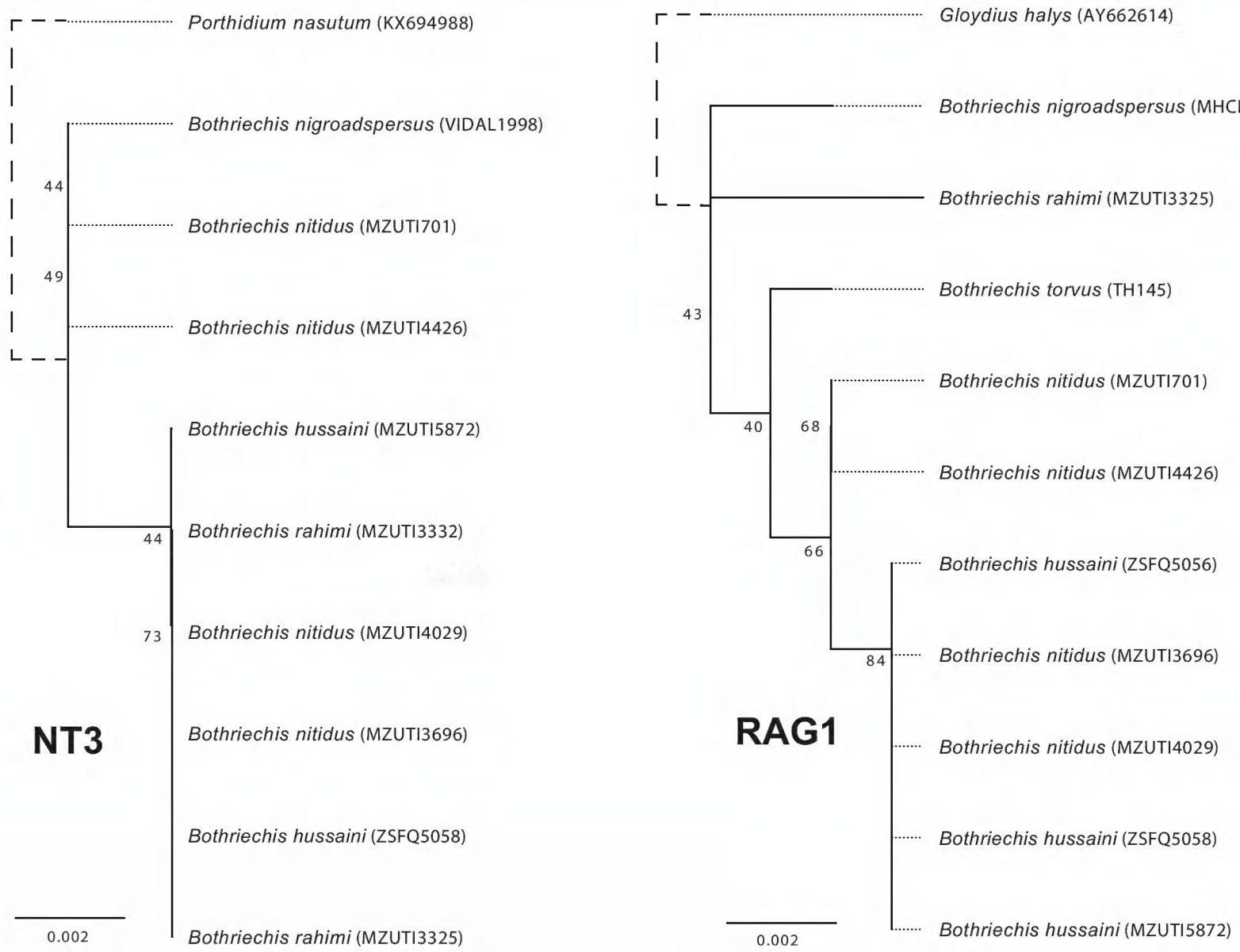


Figure 3. Phylogenetic trees derived from Bayesian inference of the nuclear genes NT3 (left) and RAG1 (right) for species of *Bothriechis*. Node labels indicate posterior probabilities reflecting support for each clade.

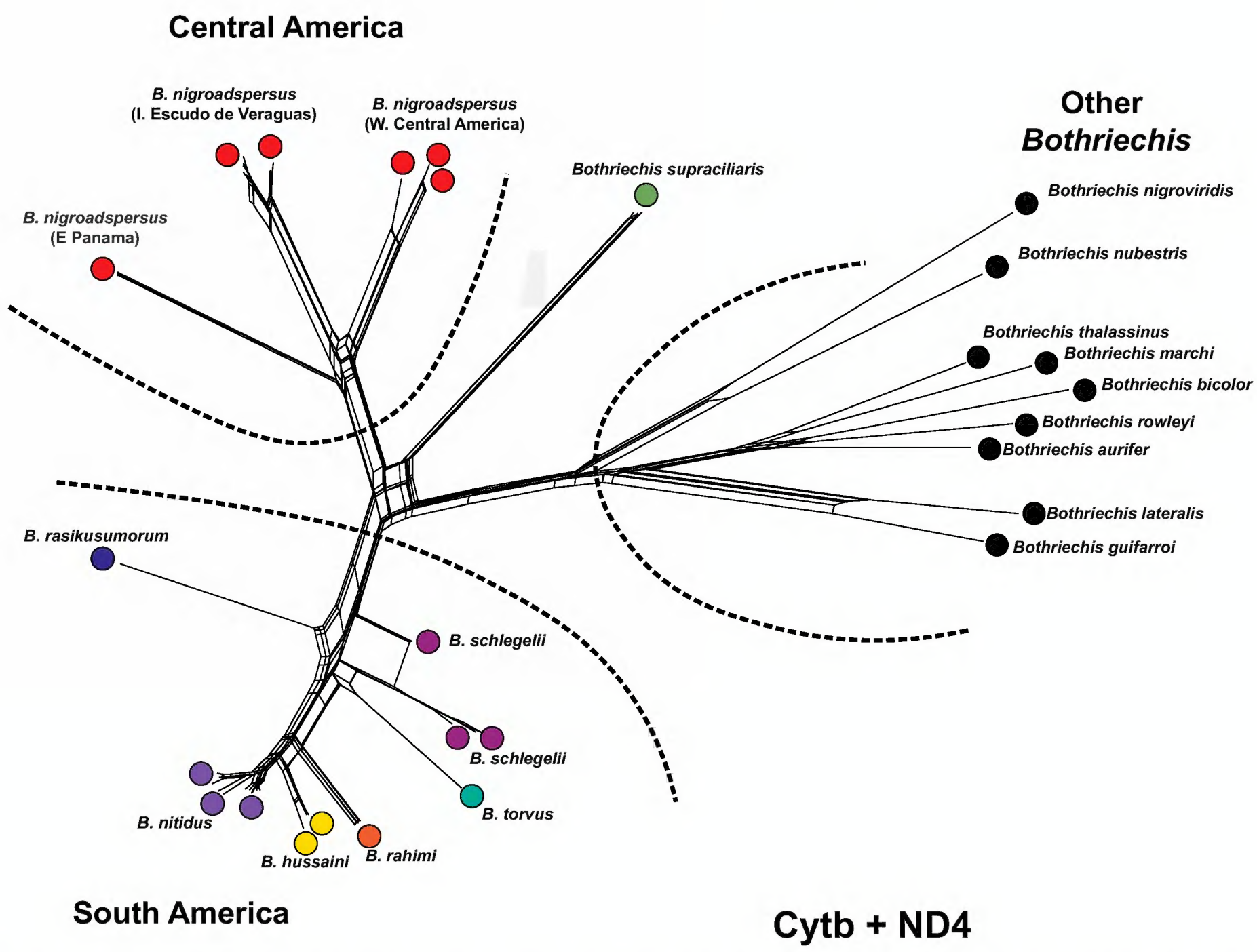


Figure 4. SplitsTree phylogenetic network of the concatenated mitochondrial DNA sequences Cytb and ND4 for members of *Bothriechis*.

Specifically, for the *B. schlegelii* species group, B. *rasikusumorum* was the sole species demonstrating consistent genetic distances above 5% when compared to the rest of the members of the group. In the case of ND4, genetic distances were generally lower than in Cytb (Table 2). Once again, only *B. rasikusumorum* had genetic distances consistently larger than 5% compared to the rest of the South American *B. schlegelii* group. Genetic distances were also generally lower in other members of *Bothriechis.* The differences in genetic distances between South American members of the *B. schlegelii* group and the Central American *B. nigroadspersus* and *B. supraciliaris* ranged between 8.0–9.8% (ND4) and 9.9–13.4% (Cytb), while the genetic differences between *B. supraciliaris* and *B. nigroadspersus* were 8.9% (ND4) and 11.5% (Cytb). These genetic differences are as high or higher than genetic distances between other members of *Bothriechis*, which could indicate species-level divergence between South and Central American populations.

The use of a 4% threshold in mtDNA for possible species-level differentiation should be treated as a way to pinpoint clades that could potentially represent distinct species and then use other sources of data to test these species' hypotheses, not as the sole evidence for species differentiation, as in the case of the study by Arteaga et al. (2024).

Table 1. Mean between-group genetic distance for *Bothriechis* species using the mitochondrial locus Cytb, expressed as a percentage.

	B. nitidus	*B. hussaini*	*B. rahimi*	*B. schlegelii*	*B. torvus*	*B. khwargi*	*B. klebbai*	*B. rasikusumorum*	**Average in *B. schlegelii* group**	*B. nigroadspersus*	*B. supraciliaris*	*B. rowleyi*	*B. aurifer*	*B. bicolor*	*B. marchi*	*B. thalassinus*	*B. guifarroi*	*B. lateralis*	*B. nubestris*	*B. nigroviridis*
B. nitidus	-	1.5	2.8	4.2	2.3	4.4	5.4	5.8	**3.8**	11.7	13.1	17.4	17.8	18.1	18.2	15.1	15.5	17.1	18.2	20.7
B. hussaini	1.5	-	2.7	4.5	1.6	5.1	5.8	6.6	**4.0**	12.1	12.7	17.9	17.4	18.0	18.2	15.4	16.2	17.2	19.3	20.9
B. rahimi	2.8	2.7	-	4.7	3.8	5.3	5.1	6.9	**4.5**	13.4	12.8	18.3	18.3	18.3	18.2	18.0	15.5	15.1	14.5	12.2
B. schlegelii	4.2	4.5	4.7	-	2.9	4.5	6.2	6.2	**4.7**	9.9	11.8	16.2	16.4	16.5	16.4	16.4	15.5	15.5	15.5	14.6
B. torvus	2.3	1.6	3.8	2.9	-	4.2	5.2	5.6	**3.7**	10.5	12.8	16.5	16.6	16.9	17.2	17.0	17.8	18.1	19.4	20.4
B. khwargi	4.4	5.1	5.3	4.5	4.2	-	4.4	6.9	**5.0**	10.6	12.5	17.0	17.1	17.2	17.2	17.1	17.9	18.7	19.8	20.5
B. klebbai	5.4	5.8	5.1	6.2	5.2	4.4	-	7.1	**5.6**	12.2	12.0	17.7	17.8	17.9	18.0	17.9	18.7	19.3	20.1	20.1
B. rasikusumorum	5.8	6.6	6.9	6.2	5.6	6.9	7.1	-	**6.4**	11.3	13.2	18.7	18.8	18.9	18.9	18.8	19.2	19.8	21.1	22.2
Average in *B. schlegelii* group	**3.8**	**4.0**	**4.5**	**4.7**	**3.7**	**5.0**	**5.6**	**6.4**												
B. nigroadspersus	11.7	12.1	13.4	9.9	10.5	10.6	12.2	11.3		-	11.5	16.9	17.0	17.2	17.3	17.1	17.7	18.6	19.8	20.3
B. supraciliaris	13.1	12.7	12.8	11.8	12.8	12.5	12.0	13.2		11.5	-	18.0	17.9	18.0	18.0	17.8	18.2	19.1	20.3	20.2
B. rowleyi	17.4	17.9	18.3	16.2	16.5	17.0	17.7	18.7		16.9	18.0	-	6.8	7.0	10.3	9.1	11.7	10.7	15.1	16.9
B. aurifer	17.8	17.4	18.3	16.4	16.6	17.1	17.8	18.8		17.0	17.9	6.8	-	7.2	9.4	7.5	11.6	11.6	16.9	16.1
B. bicolor	18.1	18.0	18.3	16.5	16.9	17.2	17.9	18.9		17.2	18.0	7.0	7.2	-	9.7	6.7	11.4	10.7	15.3	16.8
B. marchi	18.2	18.2	18.2	16.4	17.2	17.2	18.0	18.9		17.3	18.0	10.3	9.4	9.7	-	6.3	12.8	12.3	14.7	15.9
B. thalassinus	15.1	15.4	18.0	16.4	17.0	17.1	17.9	18.8		17.1	17.8	9.1	7.5	6.7	6.3	-	11.9	11.8	14.9	16.9
B. guifarroi	15.5	16.2	15.5	15.5	17.8	17.9	18.7	19.2		17.7	18.2	11.7	11.6	11.4	12.8	11.9	-	4.4	14.4	16.1
B. lateralis	17.1	17.2	15.1	15.5	18.1	18.7	19.3	19.8		18.6	19.1	10.7	11.6	10.7	12.3	11.8	4.4	-	13.7	16.6
B. nubestris	18.2	19.3	14.5	15.5	19.4	19.8	20.1	21.1		19.8	20.3	15.1	16.9	15.3	14.7	14.9	14.4	13.7	-	11.3
B. nigroviridis	20.7	20.9	12.2	14.6	20.4	20.5	20.1	22.2		20.3	20.2	16.9	16.1	16.8	15.9	16.9	16.1	16.6	11.3	-

Table 2. Mean between-group genetic distance for *Bothriechis* species using the mitochondrial locus ND4, expressed as a percentage.

	B. nitidus	*B. hussaini*	*B. rahimi*	*B. schlegelii*	*B. torvus*	*B. rasikusumorum*	**Average in *B. schlegelii* group**	*B. nigroadspersus*	*B. supraciliaris*	*B. rowleyi*	*B. aurifer*	*B. bicolor*	*B. marchi*	*B. thalassinus*	*B. guifarroi*	*B. lateralis*	*B. nubestris*	*B. nigroviridis*
B. nitidus	-	1.6	2.1	3.9	5.5	5.2	**3.7**	8.2	8.4	11.6	10.6	13.4	11.9	12.2	11.6	12.3	11.0	11.2
B. hussaini	1.6	-	2.1	4.3	5.3	5.1	**3.7**	8.5	8.6	9.8	10.5	13.3	11.5	12.1	11.5	12.6	10.6	11.0
B. rahimi	2.1	2.1	-	4.0	4.7	5.3	**3.6**	8.0	8.8	9.9	10.6	12.5	11.0	11.6	11.6	12.3	10.2	10.9
B. schlegelii	3.9	4.3	4.0	-	5.2	5.8	**4.7**	8.0	8.4	9.7	10.7	13.8	11.7	12.2	13.3	12.6	11.5	11.6
B. torvus	5.5	5.3	4.7	5.2	-	6.8	**5.5**	9.5	9.8	10.5	11.5	14.6	11.8	11.8	13.9	13.5	11.9	12.2
B. rasikusumorum	5.2	5.1	5.3	5.8	6.8	-	**5.6**	9.1	9.3	10.4	10.9	14.0	12.7	12.1	13.0	13.7	12.1	12.8
Average in *B. schlegelii* group	**3.7**	**3.7**	**3.6**	**4.7**	**5.5**	**5.6**												
B. nigroadspersus	8.2	8.5	8.0	8.0	9.5	9.1		-	8.9	10.6	13.1	16.0	14.3	14.5	15.2	14.7	13.2	13.3
B. supraciliaris	8.4	8.6	8.8	8.4	9.8	9.3		8.9	-	6.2	11.3	14.9	13.4	13.6	14.0	13.4	13.0	13.0
B. rowleyi	11.6	9.8	9.9	9.7	10.5	10.4		10.6	6.2	-	3.8	6.6	6.4	6.4	10.2	8.9	10.3	11.6
B. aurifer	10.6	10.5	10.6	10.7	11.5	10.9		13.1	11.3	3.8	-	6.7	5.9	6.2	10.5	9.6	10.2	11.9
B. bicolor	13.4	13.3	12.5	13.8	14.6	14.0		16.0	14.9	6.6	6.7	-	7.5	8.7	12.5	11.6	11.8	13.3
B. marchi	11.9	11.5	11.0	11.7	11.8	12.7		14.3	13.4	6.4	5.9	7.5	-	5.2	11.8	10.8	11.0	12.7
B. thalassinus	12.2	12.1	11.6	12.2	11.8	12.1		14.5	13.6	6.4	6.2	8.7	5.2	-	11.5	10.2	11.4	12.3
B. guifarroi	11.6	11.5	11.6	13.3	13.9	13.0		15.2	14.0	10.2	10.5	12.5	11.8	11.5	-	4.5	11.3	12.8
B. lateralis	12.3	12.6	12.3	12.6	13.5	13.7		14.7	13.4	8.9	9.6	11.6	10.8	10.2	4.5	-	11.1	11.6
B. nubestris	11.0	10.6	10.2	11.5	11.9	12.1		13.2	13.0	10.3	10.2	11.8	11.0	11.4	11.3	11.1	-	8.5
B. nigroviridis	11.2	11.0	10.9	11.6	12.2	12.8		13.3	13.0	11.6	11.9	13.3	12.7	12.3	12.8	11.6	8.5	-

Species delimitation

The species delimitation analysis conducted using DELINEATE did not support the species distinctions proposed by Arteaga et al. (2024). Instead, the maximum likelihood approach recognized only four species within the group as distinct (Fig. 5). Specifically, *B. rasikusumorum* and *B. schlegelii* were validated as separate species. Conversely, *B. khwargi* and *B. klebbai* were not differentiated and were grouped as a single taxon. Similarly, *B. torvus*, *B. rahimi*, *B. hussaini*, and *B. nitidus* were collectively treated as one taxon. Notably, the analysis's uncertainty is reflected in the variation of the 10 most probable phylogenetic trees, which identified between two and seven potential species within the *B. schlegelii* group (see Suppl. material 1 for details).

Morphological analyses

In the PCA examining morphological characters across the *B. schlegelii* group, the data did not resolve distinct clusters for the various species (Figs 6, 7, Suppl. material 3: figs S4–S9). Some clustering was observed for *B. nigroadspersus* and *B. nitidus*, but because these species represent the bulk of the individuals studied, the clusters may not signify distinct groupings but rather reflect sample size. Separate PCA analyses were conducted for scale count data and measurement data, standardized by snout-vent length (SVL) and regression residuals, for both females (Fig. 6) and males (Fig. 7). Overall, the PCA results indicated that while there is some morphological variation within the *B. schlegelii* group, particularly in *B. nigroadspersus* and *B. nitidus*, the characters examined do not provide clear distinctions between the species. The overlap in morphological traits suggests that morphological data do not differentiate between the species described by Arteaga et al. (2024).

Discussion

A recent trend in taxonomy involves grouping individuals into lineages and describing them as species without testing if the groups are evolving independently from one another (Hillis 2019). In many cases, these groupings do not consider clinal variation and are based on mtDNA data alone. The recent study of *B. schlegelii* by Arteaga et al. (2024) is one such example. Despite the authors claiming that their study follows an integration by congruence approach (Padial et al. 2010), the definition of the new or resurrected taxa in their study is mostly based on mtDNA data, as their nuclear dataset is extremely limited and does not result in monophyletic lineages (see Fig. 3).

The primary function of nuclear loci in species delimitation is to test for congruence with mtDNA and to assess

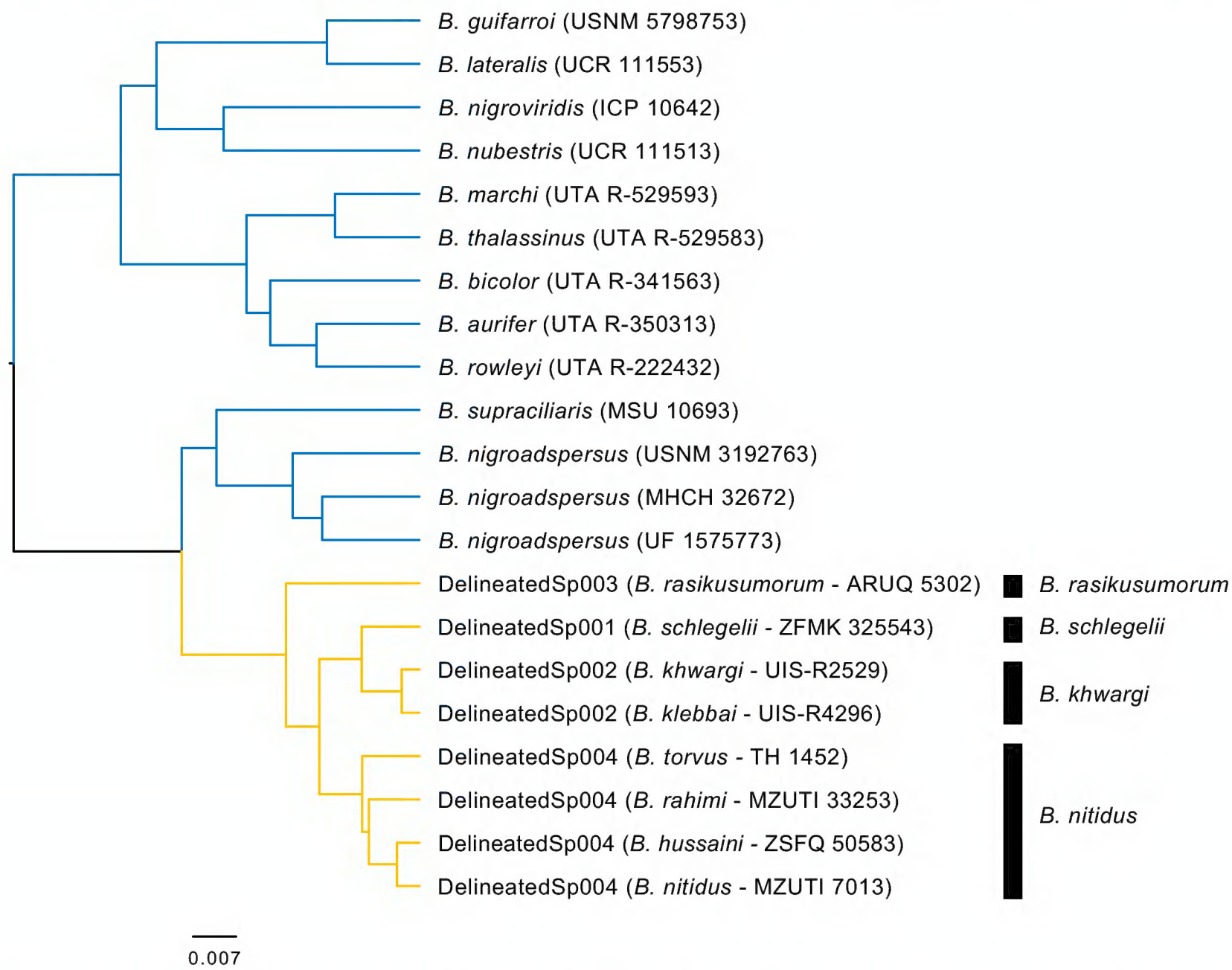

B. guifarroi (USNM 5798753)
B. lateralis (UCR 111553)
B. nigroviridis (ICP 10642)
B. nubestris (UCR 111513)
B. marchi (UTA R-529593)
B. thalassinus (UTA R-529583)
B. bicolor (UTA R-341563)
B. aurifer (UTA R-350313)
B. rowleyi (UTA R-222432)
B. supraciliaris (MSU 10693)
B. nigroadspersus (USNM 3192763)
B. nigroadspersus (MHCH 32672)
B. nigroadspersus (UF 1575773)
DelineatedSp003 (B. rasikusumorum - ARUQ 5302)
DelineatedSp001 (B. schlegelii - ZFMK 325543)
DelineatedSp002 (B. khwargi - UIS-R2529)
DelineatedSp002 (B. klebbai - UIS-R4296)
DelineatedSp004 (B. torvus - TH 1452)
DelineatedSp004 (B. rahimi - MZUTI 33253)
DelineatedSp004 (B. hussaini - ZSFQ 50583)
DelineatedSp004 (B. nitidus - MZUTI 7013)
B. rasikusumorum
B. schlegelii
B. khwargi
B. nitidus
0.007

Figure 5. Species delimitation analysis using DELINEATE. Blue clades indicate constrained species, while yellow clades denote lineages undergoing species designation testing. Terminal nodes labeled 'DelineatedSp' identify lineages that DELINEATE distinguishes as candidate separate species (sp1–4), with their former species assignations provided in parentheses. Names at right represent names that have priority.

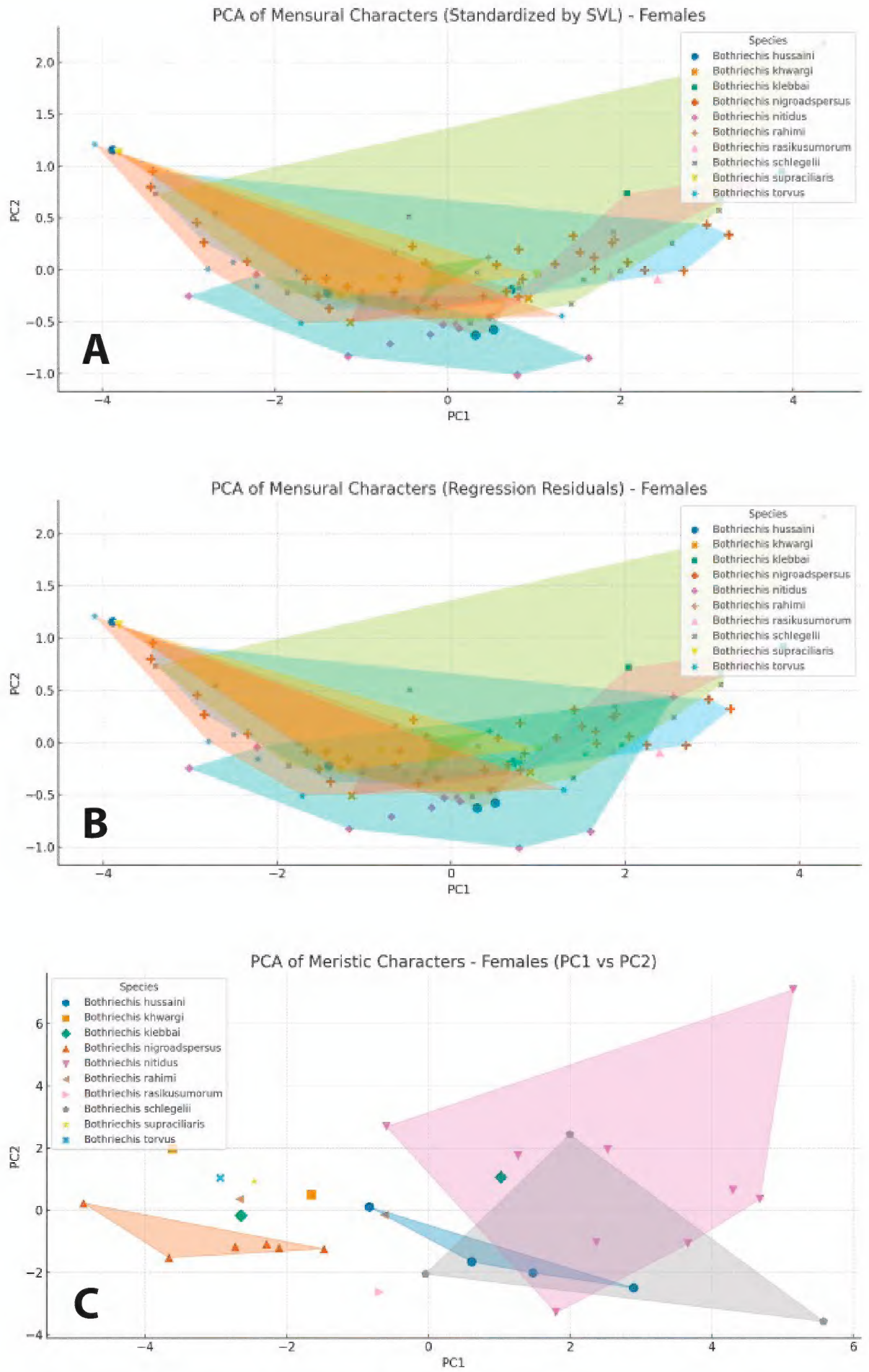
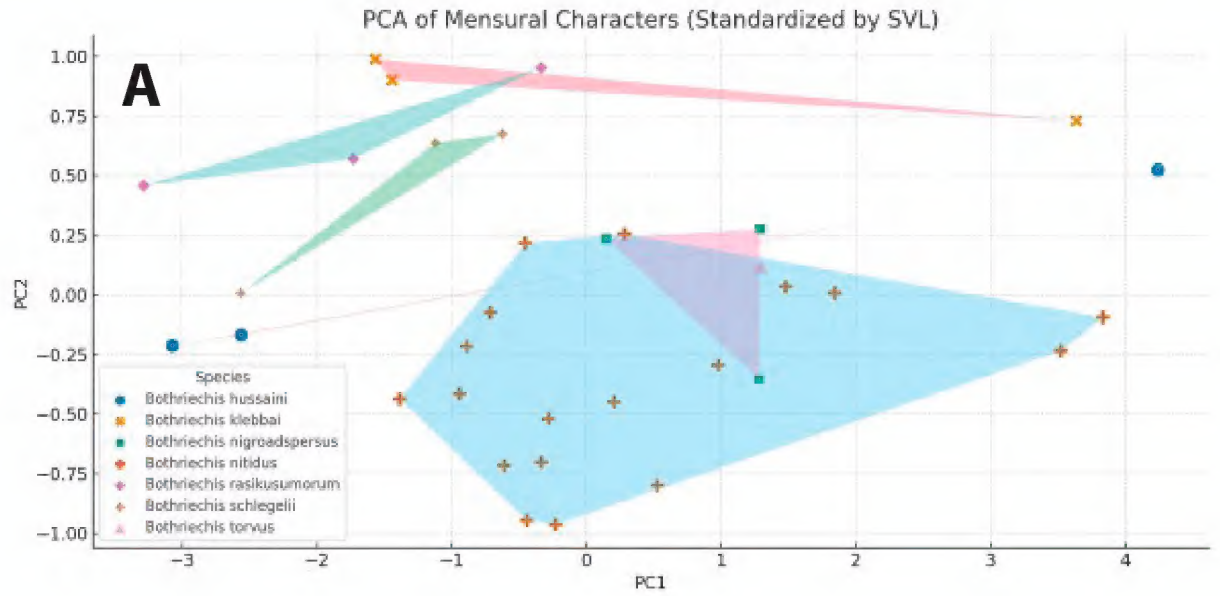


Figure 6. PCA plots for females of *Bothriechis* species. **A.** PCA of measurement characters standardized by SVL (PC1 vs. PC2), **B.** PCA of measurement characters standardized by regression residuals (PC1 vs. PC2), **C.** PCA of meristic data (PC1 vs. PC2).

genetic exchange between mtDNA-based candidate species. In species delimitation, nuclear loci should not merely serve as additional genes in a concatenated analysis. A mixed nuclear-mtDNA concatenated analysis effectively becomes a mtDNA analysis with additional noise (such as in the study of Arteaga et al. 2024) rather than a true multilocus analysis. Thus, the sequencing strategy employed by Arteaga et al. (2024), which focused heavily on mitochondrial genes and included only two nuclear genes for a small number of specimens, limits the ability to test for genetic exchange and congruence with mtDNA-based candidate species.

Although the majority of the new or resurrected species proposed by Arteaga et al. (2024) formed monophyletic groups (but not all), the relationships between the different lineages were poorly supported, likely due to incomplete lineage sorting in the loci used or rapid diversification. Furthermore, genetic distances between these putative species are very small, and genetic network visualization shows less differentiation within them than within the various populations of *B. nigroadspersus*. Only *B. rasikusumorum* displays more divergence in two mitochondrial loci than the rest of the other species in the *B. schlegelii* group, yet it was nested within other members of the group in the ML analysis (Suppl. material 3: figs S1, S2). Genetic distances of the mitochondrial genes between the Central American *B. supraciliaris* and *B. nigroadspersus* and the South American *B. schlegelii* group were quite high (8.0–13.4%), which is as high or even higher than the genetic distances between other species of *Bothriechis* (Tables 1, 2), suggesting species-level differentiation between the South and Central American populations.



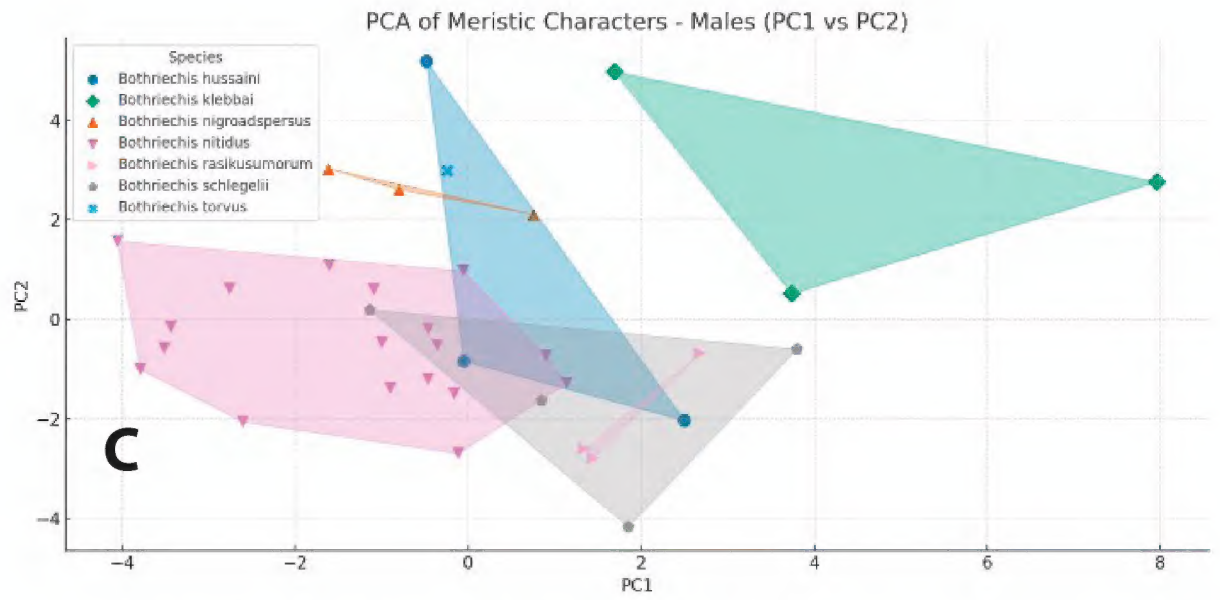


Figure 7. PCA plots for males of *Bothriechis* species. **A.** PCA of measurement characters standardized by SVL (PC1 vs. PC2), **B.** PCA of measurement characters standardized by regression residuals (PC1 vs. PC2), **C.** PCA of meristic data (PC1 vs. PC2).

A re-analysis of the morphological characters used by Arteaga et al. (2024) failed to distinguish between the multiple putative species. The study lists 22 morphological characters purported to distinguish species within the *B. schlegelii* group (table 2 in their study). Eight of these traits concern coloration, which is unreliable due to the well-documented color variation within this group. None of these color traits is exclusive to a single taxon, diminishing their discriminative power. This problem extends to scale characteristics, none of the 10 scale-related traits were found to be diagnostic, with overlaps observed among multiple species. This is particularly noticeable with ventral scales in both sexes and midbody scale rows. Furthermore, traits such as maximum total length, which are not consistent or unique to specific lineages, are not suitable for species identification.

Venom potency, measured by LD50 values, was another character considered, however, it is only available for some species. Moreover, studies by Fernandez et al. (2010) and Lomonte et al. (2012) have demonstrated significant intraspecific variation in LD50 among *Bothriechis* species, and this character is known to show intraspecific variation in other species of pitvipers (Colis-Torres et al. 2022), suggesting that these values are not species-specific.

Additionally, Arteaga et al. (2024) described the basal hooks on the hemipenial body as a distinguishing feature, but with a small sample size for most species, this trait's reliability is compromised. Only *B. schlegelii* had

a sample size greater than three, further undermining the diagnostic utility of this character. Additionally, Dowling (2002) refers to the existence of intraspecific variation in the size of these structures, indicating that they cannot reliably differentiate between species within this group. Basal hooks are known to change over the lifetime of snakes (Jadin and King 2012), with the spines (hooks) becoming more prominent with age (Lara Granados et al. 2022). Therefore, the size of the hooks is not necessarily a diagnostic character, especially when sample sizes are small. At first glance, the images of the hemipenes in Arteaga et al. (2024) might look very different from each other, especially in coloration and shape, however, the multiple colorations are the result of the preparation techniques and are not unique to each hemipenis. The same occurs with the shape of the hemipenes, as if the lobes are not completely everted, they might look more pointed (vs. rounded) at the tip. These differences are particularly noticeable in figs 15, 21, and 31 of Arteaga et al. (2024).

Lastly, the DELINEATE analysis failed to recover all species described or resurrected by Arteaga et al. (2024) (Fig. 5). Although DELINEATE has limitations when used with single-locus datasets, it was selected for its unique ability to incorporate previous taxonomic work when testing species limits (Sukumaran et al. 2021). This feature allows the integration of species concepts based on morphology, ecology, and other markers, providing a more comprehensive approach to species delimitation. However, it is important to acknowledge that this method also has its drawbacks, especially with the limited molecular dataset presented by Arteaga et al. (2024).

Previous research has shown that other single-locus species delimitation methods tend to over-split taxa (Ahrens 2024). Therefore, despite the challenges associated with using DELINEATE on a single-locus dataset, it was preferred over alternative methods to avoid artificially inflating the number of species. The results, while indicating that several of the species proposed by Arteaga et al. (2024) remain invalid, should be interpreted with caution. The data used (mtDNA) is not recommended for DELINEATE analysis, and multiple, unlinked loci should be used for this type of analysis, which are not available for this group at the present time.

Inaccurate taxonomy can dilute the already scarce conservation resources. Currently, species diversity is a major metric for biodiversity, hence, sound taxonomic practices are vital to positively impact conservation efforts and inform biological diversity studies, preventing taxonomic inflation and its associated issues. Recently, Dufresnes et al. (2023) suggested recognizing subspecies to show intra-specific diversity without resulting in taxonomic inflation. This approach could potentially be applied to some (but not all) of the clades of the *B. schlegelii* group, for example, *B. rasikusumorum*, however, if this is the case, the same should be done for the multiple populations of *B. nigroadspersus*, which I do not recommend doing with the data at hand.

Conclusion

A comprehensive review of the data challenges the validity of the taxa described or resurrected by Arteaga et al. (2024). The evidence suggests that within the *B. schlegelii* group, only *B. nigroadspersus*, *B. schlegelii*, and *B. supraciliaris* should be recognized as distinct species, however, morphological analyses failed to separate these three species. Therefore, while the current data are insufficient to definitively determine the exact number of species, it is proposed that *B. hussaini*, *B. khwargi*, *B. klebbai*, *B. nitidus*, *B. rahimi*, *B. rasikusumorum*, and *B. torvus* be considered junior synonyms of *B. schlegelii*. To fully understand the genetic relationships within the *Bothriechis schlegelii* group, future studies should incorporate comprehensive nuclear data alongside mitochondrial data to test for potential cytonuclear discordance, ensuring a more robust evaluation of species boundaries.

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References

Ahrens D (2024) Species diagnosis and DNA taxonomy. In DNA barcoding: Methods and protocols. New York, NY: Springer US., 33–52. https://doi.org/10.1007/978-1-0716-3581-0_2

Arteaga A, Pyron RA, Batista A, Vieira J, Pelayo EM, Smith EN, Amorós CLB, Koch C, Agne S, Valencia JH, Bustamante L (2024) Systematic revision of the Eyelash Palm-Pitviper *Bothriechis schlegelii* (Serpentes, Viperidae), with the description of five new species and revalidation of three. Evolutionary Systematics 8: 15–64.

Campbell JA, Lamar WW (2004) The venomous reptiles of the western hemisphere. Cornell University Press, Ithaca.

Carbajal-Márquez RA, Cedeño-Vázquez JR, Martinez-Arce A, Neri-Castro E, Machkour-M'Rabet SC (2020) Accessing cryptic diversity in Neotropical rattlesnakes (Serpentes: Viperidae: Crotalus) with the description of two new species. Zootaxa 4729(4): 451–481. https://doi.org/10.11646/zootaxa.4729.4.1

Chambers EA, Hillis DM (2020) The multispecies coalescent oversplits species in the case of geographically widespread taxa. Systematic Biology 69: 184–193. https://doi.org/10.1093/sysbio/syz042

Colis-Torres A, Neri-Castro E, Strickland JL, Olvera-Rodríguez A, Borja M, Calvete J, Jones J, Parkinson CL, Bañuelos J, de León JL, Alagón A (2022) Intraspecific venom variation of Mexican West Coast Rattlesnakes (*Crotalus basiliscus*) and its implications for antivenom production. Biochimie 192: 111–124. https://doi.org/10.1016/j.biochi.2021.10.006

DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philosophical Transac-

tions of the Royal Society B: Biological Sciences 360(1462): 1905–1916. https://doi.org/10.1098/rstb.2005.1722

Dowling HG (2002) Intraspecific variation of the hemipenis, a correction, with comments on other erroneous descriptions. Herpetological Review 33(1): 12.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 1–8. https://doi.org/10.1186/1471-2148-7-1

Dufresnes C, Poyarkov N, Jablonski D (2023) Acknowledging more biodiversity without more species. Proceedings of the National Academy of Sciences 120(40): e2302424120. https://doi.org/10.1073/pnas.2302424120

Fernández J, Lomonte B, Sanz L, Angulo Y, Gutiérrez JM, Calvete JJ (2010) Snake venomics of *Bothriechis nigroviridis* reveals extreme variability among palm pitviper venoms: different evolutionary solutions for the same trophic purpose. Journal of Proteome Research 9(8): 4234–4241. https://doi.org/10.1021/pr100545d

Folt B, Bauder J, Spear S, Stevenson D, Hoffman M, Oaks JR, Wood Jr PL, Jenkins C, Steen DA, Guyer C (2019) Taxonomic and conservation implications of population genetic admixture, mito-nuclear discordance, and male-biased dispersal of a large endangered snake, *Drymarchon couperi*. PLoS ONE 14(3): e0214439.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics 34: 397–423. https://doi.org/10.1146/annurev.ecolsys.34.011802.132421

Hebert PDN, Cywinska S, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Hillis DM (2019) Species delimitation in herpetology. Journal of Herpetology 53(1): 3–12. https://doi.org/10.1670/18-123

Hurtado-Gómez JP (2009) Patrones de variación morfológica de *Bothriechis schlegelii* (Serpentes: Viperidae) en un gradiente altitudinal. BSc thesis, Universidad de Antioquia.

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23(2): 254–267. https://doi.org/10.1093/molbev/msj030

Jadin RC, King RB (2012) Ontogenetic effects on snake hemipenial morphology. Journal of Herpetology 46(3): 393–395. https://doi.org/10.1670/11-237

Jadin RC, Blair C, Orlofske SA, Jowers MJ, Rivas GA, Vitt LJ, Ray JM, Smith EN, Murphy JC (2020) Not withering on the evolutionary vine: systematic revision of the Brown Vine Snake (Reptilia: Squamata: *Oxybelis*) from its northern distribution. Organisms Diversity & Evolution 20: 723–746. https://doi.org/10.1007/s13127-020-00461-0

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108

Kück P, Longo GC (2014) FASconCAT-G: extensive functions for multiple sequence alignment preparations concerning phylogenetic studies. Frontiers in Zoology 11: 1–8. https://doi.org/10.1186/s12983-014-0081-x

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Krysko KL, Granatosky MC, Nuñez LN, Smith DL (2016) A cryptic new species of indigo snake (genus *Drymarchon*) from the Florida platform of the United States. Zootaxa 4138: 549–569. https://doi.org/10.11646/zootaxa.4138.3.9

Lara Granados G, Greenwood J, Secor S, Shan S, Hedrick BP, Brennan PL (2022) Examining the shape and size of female and male genitalia in snakes using three-dimensional geometric morphometrics. Biological Journal of the Linnean Society 136(3): 466–476. https://doi.org/10.1093/biolinnean/blac051

Lomonte B, Tsai WC, Bonilla F, Solórzano A, Solano G, Angulo Y, Calvete JJ (2012) Snake venomics and toxicological profiling of the arboreal pitviper *Bothriechis supraciliaris* from Costa Rica. Toxicon 59(5): 592–599. https://doi.org/10.1016/j.toxicon.2012.01.005

Miller MA, Pfeiffer W, Schwartz T (2011) The CIPRES science gateway: a community resource for phylogenetic analyses. In: Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery, 8 pp. https://doi.org/10.1145/2016741.2016785

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Padial JM, Miralles A, de la Riva I, Vences M (2010) The integrative future of taxonomy. Frontiers in Zoology 7: 16. https://doi.org/10.1186/1742-9994-7-16

Rambaut A (2014) FigTree v1. 4.2. http://tree.bio.ed.ac.uk/software/figtree/

Rambaut A, Suchard M, Drummond AJ (2022) Tracer version 1.7.1. http://www.beast.community

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sukumaran J, Knowles LL (2017) Multispecies coalescent delimits structure, not species. Proceedings of the National Academy of Sciences 114: 1607–1612. https://doi.org/10.1073/pnas.1607921114

Sukumaran J, Holder MT, Knowles LL (2021) Incorporating the speciation process into species delimitation. PLoS Computational Biology 17: e1008924. https://doi.org/10.1371/journal.pcbi.1008924

Tepos-Ramírez M, Flores-Villela O, Velasco JA, Pedraza Lara C, García Rubio OR, Jadin RC (2021) Molecular phylogenetics and morphometrics reveal a new endemic jumping pitviper (Serpentes: Viperidae: *Metlapilcoatlus*) from the Sierra Madre Oriental of Mexico. Journal of Herpetology 55(2): 181–191. https://doi.org/10.1670/20-028

Thorpe RS (1975) Quantitative handling of characters useful in snake systematics with particular reference to intraspecific variation in the ringed Snake *Natrix natrix* (L.). Biological Journal of the Linnean Society 7: 27–43. https://doi.org/10.1111/j.1095-8312.1975.tb00732.x

Thorpe RS (1983) A review of the numerical methods for recognizing and analyzing racial differentiation. In: Felsenstein J (Ed.) Numerical Taxonomy. NATO ASI Series. Vol. 1. Springer, Berlin, Heidelberg, pp. 404–423. https://doi.org/10.1007/978-3-642-69024-2_43

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44(W1): W232–W235. https://doi.org/10.1093/nar/gkw256

Wylie DB, Grünwald CI (2016) First report of *Bothriechis schlegelii* (Serpentes: Viperidae: Crotalinae) from the state of Oaxaca, Mexico. Mesoamerican Herpetology 3.4: 1066–1067.

Supplementary material 1

Results of the DELINEATE analysis

Author: Jacobo Reyes-Velasco

Data type: trees

Explanation note: The file presents the ten most probable species assignations, ranked in descending order of likelihood.

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Link: https://doi.org/10.3897/herpetozoa.37.e131965.suppl1

Supplementary material 2

Supplementary tables

Author: Jacobo Reyes-Velasco

Data type: xlsx

Explanation note: **table S1.** Principal component loadings for measurement characters standardized by SVL in females of *Bothriechis* species. **table S2.** Explained variance for principal components of measurement characters standardized by SVL in females of *Bothriechis* species. **table S3.** Principal component loadings for measurement characters standardized by SVL in males of *Bothriechis* species. **table S4.** Explained variance for principal components of measurement characters standardized by SVL in males of *Bothriechis* species. **table S5.** Principal component loadings for measurement characters standardized by regression residuals in females of *Bothriechis* species. **table S6.** Explained variance for principal components of measurement characters standardized by regression residuals in females of *Bothriechis* species. **table S7.** Principal component loadings for measurement characters standardized by regression residuals in males of *Bothriechis* species. **table S8.** Explained variance for principal components of measurement characters standardized by regression residuals in male *Bothriechis* species. **table S9.** Principal component loadings for meristic characters in females of *Bothriechis* species. **table S10.** Explained variance for principal components of meristic characters in females of *Bothriechis* species. **table S11.** Principal component loadings for meristic characters in males of *Bothriechis* species. **table S12.** Explained variance for principal components of meristic characters in males of *Bothriechis* species.

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Supplementary material 3

Supplementary figures

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Data type: docx

Explanation note: **figure S1.** Maximum likelihood phylogenetic tree derived from all seven nuclear and mitochondrial concatenated loci using IQ-TREE. Bootstrap support values are displayed at the nodes. **figure S2.** Maximum likelihood phylogenetic tree derived from five mitochondrial concatenated loci using IQ-TREE. Bootstrap support values are displayed at the nodes. **figure S3.** Phylogenetic relationships of *Bothriechis* species based on Bayesian inference from concatenated mitochondrial loci. Node numbers indicate posterior support values. **figure S4.** PCA of measurement characters standardized by SVL in females of *Bothriechis* species (PC1 vs. PC3). **figure S5.** PCA of measurement characters standardized by SVL in males of *Bothriechis* species (PC1 vs. PC3). **figure S6.** PCA of measurement characters standardized by regression residuals in females of *Bothriechis* species (PC1 vs. PC3). **figure S7.** PCA of measurement characters standardized by regression residuals in males of *Bothriechis* species (PC1 vs. PC3). **figure S8.** PCA of meristic data in females of *Bothriechis* species (PC1 vs. PC3). **figure S9.** PCA of meristic data in males of *Bothriechis* species (PC1 vs. PC3).

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